Soil Aggregates as Microhabitats of Microorganisms

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(Received November 4, 1987)

I Introduction

The life and behaviour of soil microorganisms are largely affected by various factors of their immediately surrounding environment. As well known soil is a very heterogeneous system and it seems very complicated to analyze the effect of each factor on microbial cells living at different sites of soil. However we can expect that the problem can be simplified and the analysis of these factors may become possible to some extent by introducing a model of their microhabitats. The aggregate structure may be useful for this purpose and, in this review, we will try to analyze microbial distribution and their behaviour in relation to aggregate structure.

II Fractionation of microbial cells in soil aggregates

When we examine experimentally the distribution of microbial cells in aggregates, there may be two kinds of approach; one is the fractionation of microbial cells by some procedure which may be able to distinguish cells existing at different sites of aggregates; the other is microscopic or electron microscopic observation of microbial cells in situ. In this review we will mainly consider the results of fractionation experiment.

Tyagny-Ryadno (1962) studied microbial distribution by treating dried soil aggregates with collodion so that each aggregates were covered with a thin layer of this material; the treated aggregates were then shaken in water for about 5 minutes, since organisms in the outer part was floated to the surface of water. The organisms remaining in soil were considered those in the inner part. Bacteriological studies were carried out on bacteria in both collodion-treated and non-treated soil samples and thus the microbial content in the outer part was determined by difference. The method showed differences in bacterial constituents between the two parts, but collodion had to be dissolved in a mixture of ethanol and ether, both of which must be toxic to microbial cells.

Hattori (1967, 1973) proposed another method called "washing-sonication method", which relies on an idea that water stable aggregates can be washed gently to remove microbial cells from the outer part and those in the inner part can be dispersed by sonic oscillation. The following experiments will support the idea.

1. Washing-out of microbial cells from aggregates

It is generally difficult to disperes fully microbial cells in soil aggregates into an aqueous medium. However it does not mean that all the cells are not easily released from aggregates, but some of them do so very easily. Now let us put one gram of aggregates into a sterilized 200 *ml* Erlenmeyer

Rep. Inst. Agr. Res. Tohoku Univ., 37: 23-36. 1988.
The 525th report of the Institute for Agricultural Research, Tohoku University (March, 1988)

flask and pour 100 *ml* of sterilized water taking care not to disrupt aggregates mechanically by water drops. After having shaken gently the flask we stand by it for 5 minutes and then remove the supernatant into another sterilized flask. We repeat the same procedure several times or more, and then treat the remaining aggregates by sonic oscillation to disperes them. Then we count the number of cells in each washing and the sonicated suspension by the plating method or the most probable number method.

Figures 1-3 show some typical results of such washing experiments. The numbers of bacteria, fungi and protozoa in each washing decreased very sharply by repeated washing. By disruption of aggregates by sonic oscillation a large number of bacterial cells were dispersed but protozoa released by this treatment were so small in number. A moderate number of fungi was found in the sonicated suspension.

We may consider that microbial cells in soil aggregates can be divided into two fractions; those easily washed out (W fraction) and those retained in aggregates against washing (R fraction). Generally bacteria and fungi can be founnd in both the fractions but protozoa mainly in the W fraction.

Next we shall study the difference of bacte-

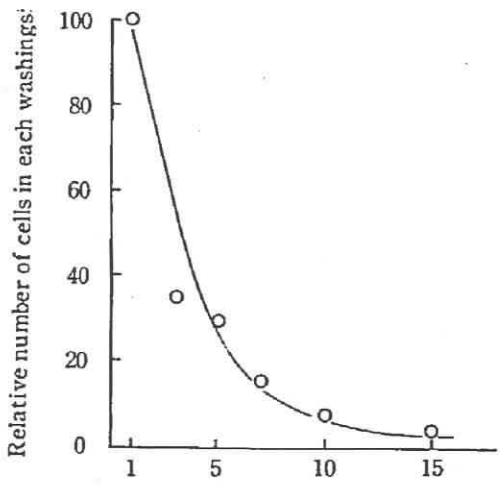

Figure 1. Washing out of bacterial cells in aggregates.
The number of bacterial cells which dispersed by sonic oscillation after washing was 30 to 40 times of that of cells in the first washings. Hattori, 1967.

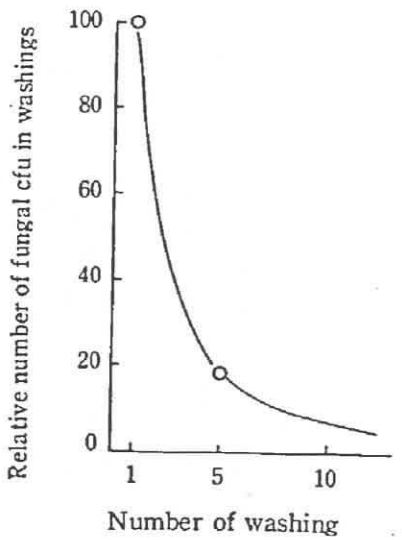

Figure 2. Washing out of fungi in aggregates.
The number of fungal colony forming unit which dispersed by sonic oscillation was 10 to 20% of that in the first washings.

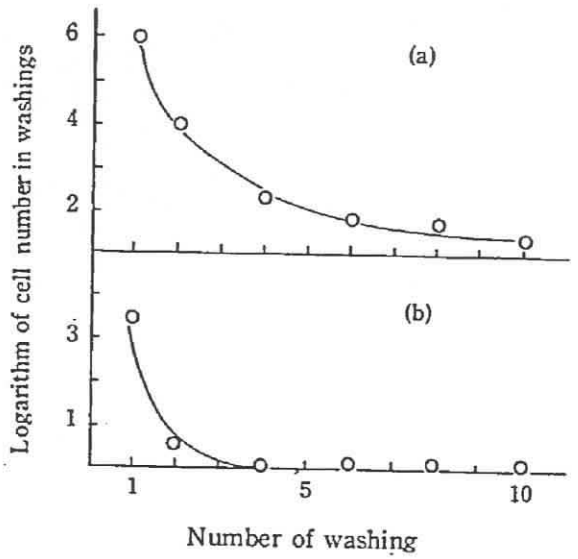

Figure 3. Washing out of protozoan cells in aggregates.
(a); Amoeba. By sonic oscillation 10^4 cells were newly dispersed. (b); Colpoda. By sonic oscillation any cells was hardly observed to dispersed newly into water. Vargas and Hattori, to be published.

rial constituents between the two fractions and also of the effects of drying and toxic substances on the viability of bacteria in these fractions.

2. Gram negative bacteria in soil population

It is generally accepted that, in soil, Gram positive bacteria are more abundant than Gram negative ones. But, according to our fractionation experiment, it is the case only with bacteria in the W fraction. Bacterial population in the R fraction involves Gram negative bacteria as many as or more than Gram positive ones.

3. Effect of air-drying on the viability of bacteria

Air-drying results in the decrease of the number of bacteria. But it is notable here that air-drying affects more severely the viablity of bacteria in the W fraction than those in the R fraction. Figure 4 shows that, when aggregates were percolated with a 20*mM* glycine solution, a larger increase in the bacterial number was observed in the W

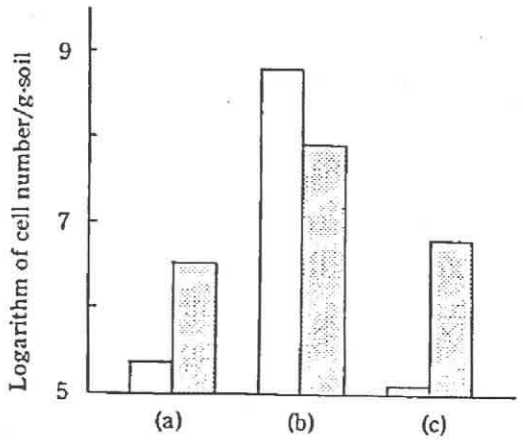

Figure 4. Fractionation of bacterial cells in aggregates under various conditions.
(a); air-dried soil, (b); soil percolated with 20 *mM* glycine solution for 62 *hrs*, and (c); soil (b) was air-dried again. White and shadowed columns indicate the W and the R fractions, respectively.

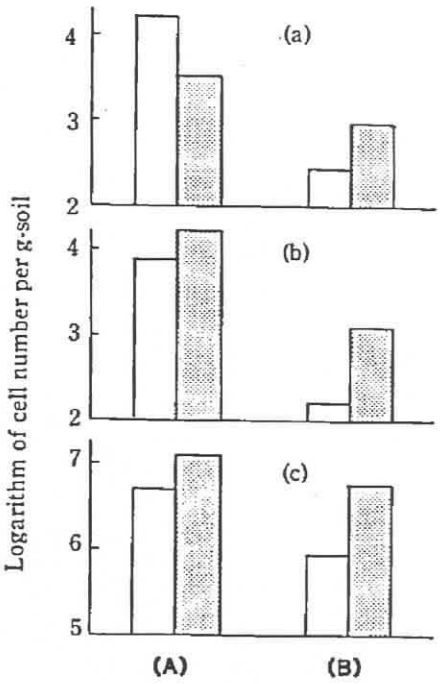

Figure 5. Fractionation of nitrifying bacteria in aggregates.
(a); ammonium oxidizer, (b); nitrite oxidizer, and (c); heterotrophic bacteria. (A); soil percolated with 10 *mM* ammonium sulphate solution, and (B); the above soil was desiccated over P_2O_5 in a desiccator. Nishio and Furusaka, 1970.

fraction and a more sever decrease in the number occured in the W fraction by air-drying than in the R fraction. We can consider that the sites at which cells in the W fraction have existed lose water more easily by air-drying than the sites surrounding cells in the R fraction.

Similar results were also obtained with aggregates percolated with an ammonium salt solution (Figure 5).

These results were interpreted that cells in the W fraction were released from larger pores while those in the R fraction were retained in smaller pores.

4. Effects of toxic substances on the viability of bacteria

We can also recognize the sites at which cells of each fraction have resided by examining the effects of toxic substances on their

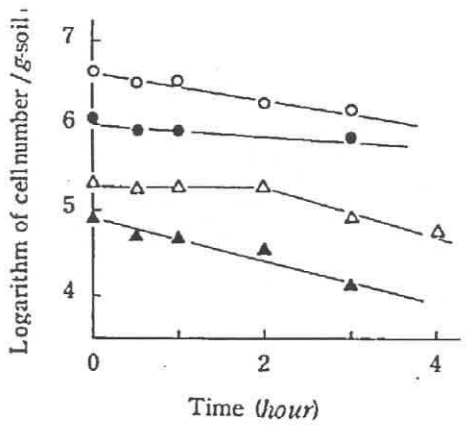

Figure 6. Effect of ethylene dibromide gas on bacterial population in aggregates.
Opened and closed circles indicate total bacteria in the R and the W fractions, respectively. Opened and closed triangles indicate Gram negative bacteria of the R and the W fractions, respectively. Hattori, 1967.

viability.

First we consider the effect of ethylene dibromide; one gram of soil aggregates (0.8–2 *mm* in diameter) was put into the main chamber of each of Thunberg tubes and an amount of liquid ethylene dibromide in the side chamber and then kept at 16*C*. The substance volatilized and diffused into aggregates. At intervals tubes were taken out one by one to count the number of bacteria in the W and R fractions. A remarkable decrease was recognized not in the number of the total bacteria which was estimated using the nutrient broth medium, but in the number of Gram negative bacteria which was estimated using the medium containing 5×10^{-4}% crystalviolet (Figure 6); the number of Gram negative bacteria began to decrease as soon as the experiment was started with the W fraction and did so after 2 *hrs* with the R fraction. In this experiment most Gram positive bacteria were probably in spore forms resistant to the substance.

More remarkable effects were obtained under $HgCl_2$ treatment; soil aggregates were treated with various concentrations of $HgCl_2$

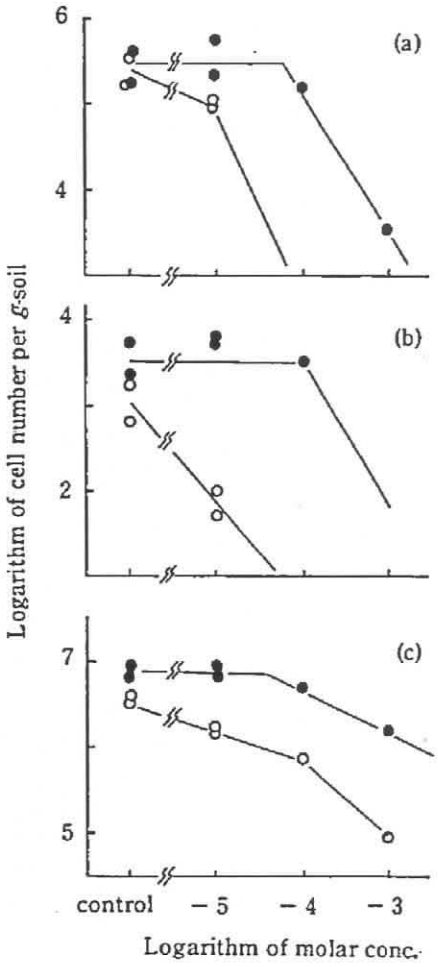

Figure 7. Effect of $HgCl_2$ on nitrifying bacteria in aggregates.
Soil aggregates were, after being air-dried, percolated with 10 *mM* ammonium salt solution for 18 days was used. (a); ammonium oxidizer, (b); nitrite oxidizer, and (c) heterotrophic bacteria. Opened and closed circles indicate bacterial number in the W and the R fractions, respectively. See text. Nishio and Furusaka, 1970.

for 30 *min* at 25*C* and then cells were fractionated to estimate their numbers respectively. In the experiment aggregates percolated with an ammonium salt solution were used and the numbers of ammonium and nitrate oxidizers were estimated by the most probable method and the number of heterotrophic bacteria by plate counting. As shown in Figure 7 the number of each bacterial group decreased markedly by a more diluted $HgCl_2$ with the W fraction than with the R fraction. These results confirm that

cells in the W fraction are more easily affected by exogeneously added inhibitor than those in the R fraction. That is, inhibitor molecules are more easily accesible to the sites at which cells of the W fraction have existed than to the site of those in the R fraction.

III Distribution of microbial cells in aggregates

Here we shall attempt to make up a model defining the distribution of microbial cells in aggregates and their dynamics based on the above results of the fractionation experiments. Two factors seem promising for our purpose, that is, adhesion of microbial cells onto soil particles and the size of pores in which cells are residing.

1. Adhesion of cells onto soil particles

One may suppose that cells in the R fraction may have adhered closely to soil particles and, therefore, they were not washed out. On the contrary cells in the W fraction may have been suspended in soil solution freely or attached weakly to soil particles and, consequently, they were easily washed out.

This idea requires that, in the case of field soil before and after air-drying, Gram negative bacteria should adhere more strongly to soil particles than Gram positive bacteria. But, in the case of precolated soil, such selective adhesion is not required; Gram negative bacteria are predominant in both the fractions.

Undoubtedly the surface of soil particles affects the behaviour of microbial cells, especially, those existing in small pores. But the effect may not be competent for the simplified understanding of microbial distribution and behaviour. Then we will note another factor, the size of pores in which cells are living. The size of pores will be discussed in relation to the size of microbial cells and water retention of the pores.

2. Pore size as a limiting factor of cell distribution

Bacterial cells in soil are considered of a size ranging from 0.2 to 1.5 μm. On the other hand the width of fungal phyphae is wider than several μm and the size of conidiospores are larger than about 5 μm; the size of protozoan and algal cells are also larger than several micron meters.

Now we can assume that the sites at which cells of the R fraction exist are pores, the diameter of which may be smaller than about 5 μm and that cells existing in pores larger than 5 μm in diameter may be easily washed out (W fraction). We will call the former pores the outer part of aggregates and the latter the inner part of aggregates.

Based on the assumption bacterial cells are considered to exist in both parts, but fungal, protozoan and algal cells exist mainly in the outer parts.

3. Water retention and pore size

Another aspect of pore size is related to the retention of water necessary for microbial life. Soil water is often classified on the base of relative degree of retention, simply under three heads; free, capillary and hygroscopic. For the purpose of simplification, we shall concentrate our attention on the capillary water, the tension of which varies from 0.1 to 31 atmosphere. The retention can be expressed in terms of pF value proposed by Schofield (1935) and the pF value can be roughly connected with the diameter of pore neck as follows (Smart, 1975);

$$pF = \log 0.3 - \log d \qquad (1)$$

where d is the diameter of pore neck in centimeter. Pores in aggregates can be

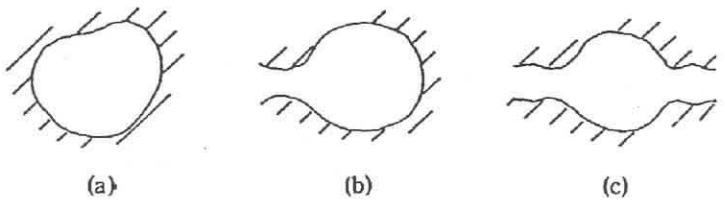

Figure 8. Various types of pores.
(a); Closed pore, (b); bottle-type pore, and (c); tubular pore with opened ends.

classified into three types (Figure 8); tubular pores with narrow opened ends, bottle pores and closed pores.

We can roughly estimate the size of pore neck of the latter two types using the above equation. Assuming the diameter of a pore to be 1.5 or more times of that of the pore neck, we can also estimate, though very roughly, the pore size.

4. Estimating of the critical pore size between the outer and inner parts.

Using the relation between water retention and pore size we can estimate the critical pore size dividing pores into the outer and inner parts. Previously we (Hattori and Hattori, 1976) tried to make an estimate from the fact that the number of bacteria increased proportionally to water content of soil until a critical value of water content. With a larger water content than the value, the number was not affected distinctly by water content (Figure 9). Although the critical water content was specific to each soil, *pF* value corresponding to the critical value was between 2.7 and 3.0. The result of this estimate revealed that the critical size was between 3 and 6 μm in diameter. Although we did not refer it the value was the diameter of pore neck and therefore the diameter of pore should be 1.5 or more times.

Now we will make another approach. Soil aggregates were, after being air dried, sterilized by autoclaving and inoculated a definite volume of cell suspension of *E. coli*.

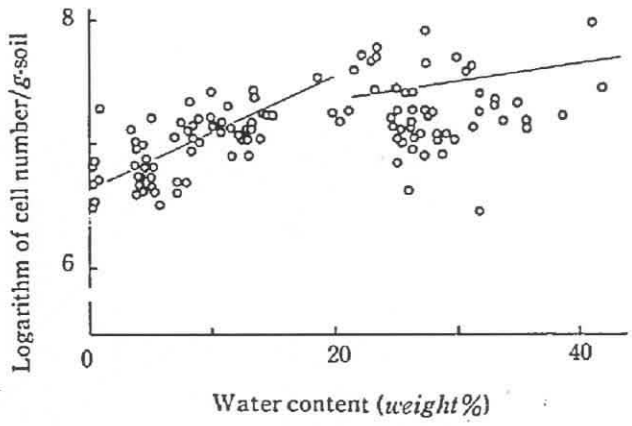

Figure 9. Relation between bacterial number and water content of soil.
Tanaka and Sakamoto, 1972.

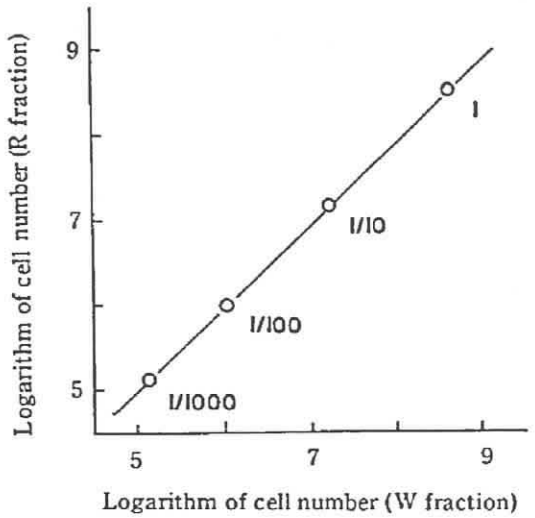

Figure 10. Partition of *E. coli* cells into the W and the R fractions.
Sterilized aggregates were used. Fractional number in figure indicates the degree of dilution of cell suspension added. The standard cell suspension contained 3.8×10^8 cells/*ml* and the volume of added supension was 0.25 *ml*/*g*-soil. Hattori, 1967.

Then the cells were fractionated by the washing-sonication method.

As shown in Figure 10, the ratio of the number of cells of the W fraction to that of cells of the R fraction was constant throughout the experiments, though different concentrations of cell suspension were used.

This result means that, since the volume of added cell suspension is constant, cells move into pores in the inner part with water. From the ratio we can estimate the volume of cell suspension that filled the pores of the inner part and then the critical pore size using the water content-pF relation and equation (1); the value thus obtained was 2.5 μm in diameter of pore-neck.

IV Dynamics of bacterial population in soil aggregates

We have studied the dynamic feature of bacterial population in aggregates by the washing-sonication method. Here we refer five examples.

1. Aggregates percolation with glycine solution

Soil aggregates were after being air dried, percolated with 20 mM glycine. The number of bacteria increased in both parts of aggregates, but the degree of increase was larger in the outer part than in the inner part (Figure 4 and 11). When percolated aggregates were air dried, the number of bacteria decreased more remarkably in the outer part than in the inner part (Figure 4).

2. Aggregates percolation with ammonium salt or nitrite solution

Similar results were also obtained with aggregates percolated with ammonium salt or nitrite solution, in which ammonium oxidizers and/or nitrite oxidizers proliferateted together with heterotroph (Figure 12).

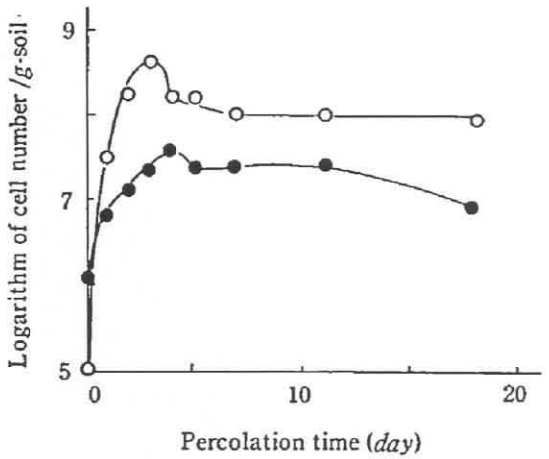

Figure 11. Increase in the number of bacteria in aggregates during percolation with 20 mM glycine solution.
Opened and closed circles indicate bacteria in the outer and the inner part of aggregates. Nioh and Furusaka, 1969.

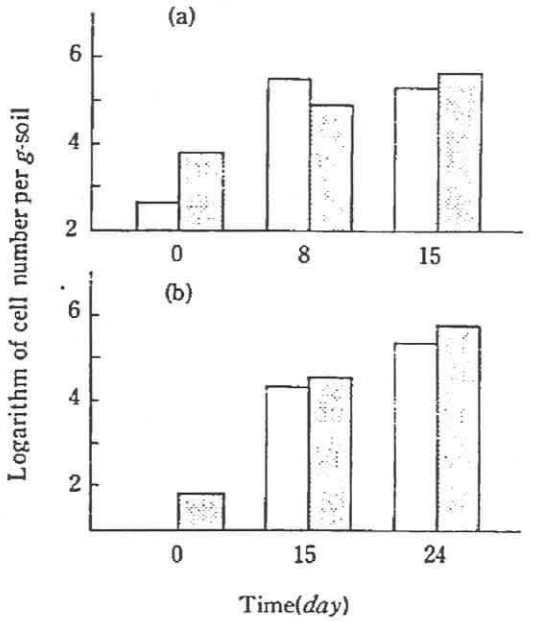

Figure 12. Increase in the number of bacteria in aggregates during percolation with 10 mM ammonium salt or 10 mM nitrite salt solution.
(a); ammonium oxidizer in soil percolated with ammonium salt solution, (b); nitrite oxidizer in soil percolated with nitrite solution. White and shadowed columns indicates, respectively, the bacteria in the outer and the inner parts. Nishio and Furusaka, 1970.

3. Distribution of added cells in sterilized aggregates

If our interpretation of distribution of added cells is acceptable, we may expect the partition ratio of cells into the inner part becomes smaller if sterilized water is added before cell suspension. Figure 13 shows such a sharp decrease in the bacterial number of the inner part and a gradual decrease in the number of the outer part. Therefore we can conclude that, at first, water moves into smaller pores with cells because the capillary force is stronger in smaller pores.

4. Oxygen consumption by cells in aggregates

Washed cell suspension of *E. coli* was added into sterilized aggregates and, using glucose as a substrate, oxyen uptake by the cells was observed in a buffer solution of *pH* 6.0. In this experiment we controlled the number of cells by the concentration and the volume of cell suspension.

The rate of oxygen consumption increased with the number of cells. We should note here that the maximal rate was higher when cells were distributed in the outer part as well as in the inner part than when cells were distributed mainly in the inner part. In the former case the rate reflected mainly the maximal value of cells in the outer part as shown in Figure 14. The result indicated that cells in the inner part exist in smaller pores than those in the outer part.

Notably the maximum rate of cells in the inner part was larger in the case of a volcanic soil which is more porous than in the case of an aluvial soil which is less porous.

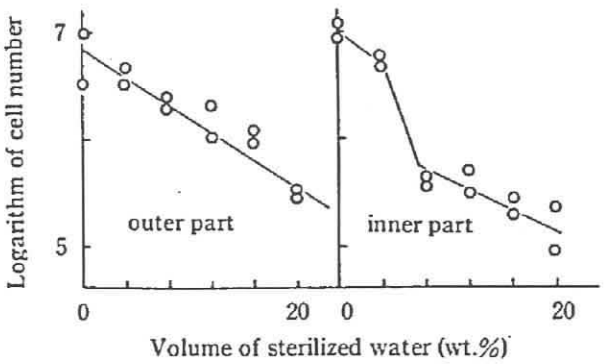

Figure 13. Effect of the addition of sterilized water before the addition of cell suspension into sterilized aggregates on the partition of cells into the inner and the outer parts.

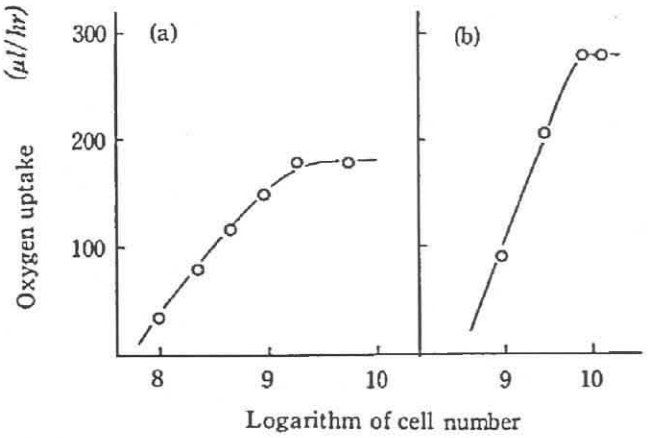

Figure 14. O_2 uptake by *E. coli* cells in the inner (a) and the outer (b) parts per g-soil. Glucose was used as the substrate. In the case of the outer part the number of cells indicates the sum of that in the outer and the inner parts. Hattori, 1967.

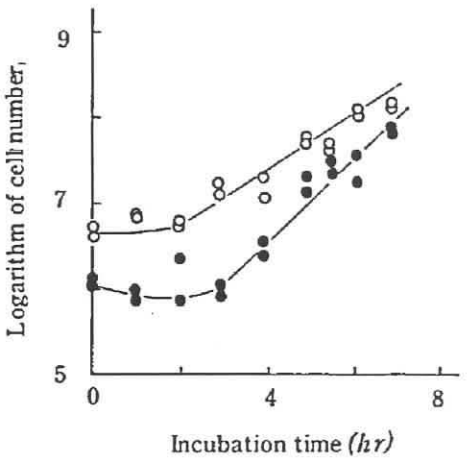

Figure 15. Growth of *E. coli* in sterilized aggregates.
Opened and closed circles indicate growth in the outer and the inner parts. Cells were suspended in the nutrient broth and 0.25 *ml* per gram soil of the suspension was added. Hattori, 1967.

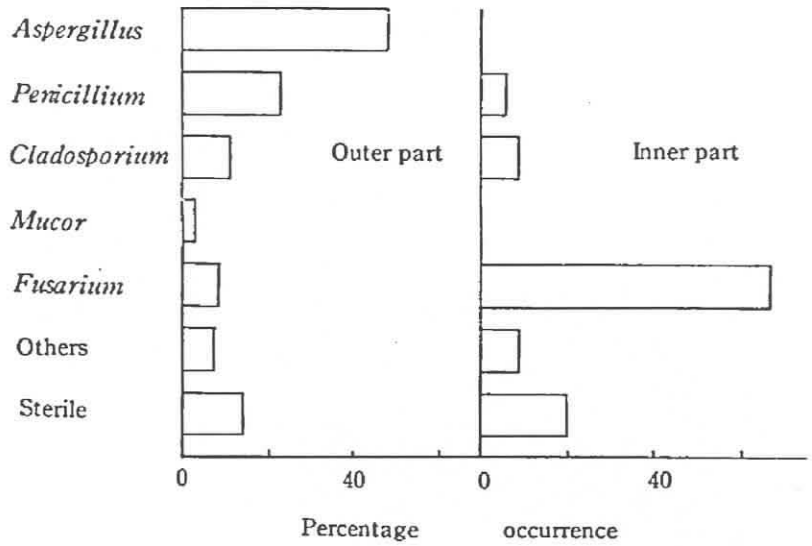

Figure 16. Fungal distribution in aggregates.

5. Growth of cells in sterilized aggregates

When cells suspended in the nutrient broth were added into sterilized aggregates and incubated, the numbers of cells in the outer and inner parts increased, respectively, showing different growth curves (Figure 15). The result again indicates that cells in the two parts are living in different environments.

V Distribution of fungi in aggregates

Most of fungi were found in the W fraction, indicating that they were living in the outer part and that only a small part of them were in the inner part. Figure 16 shows that the fungal group in the inner part was different from that in the outer part ; in the inner part *Fusarium* spp. were dominat.

VI Distribution and predation behaviour of protozoa

We analyze, here, the distribution and predation behaviour of protozoa in aggregates.

1. Distribution of various protozoa among aggregates

We examined the frequency of occurence of various protozoa groups in aggregates (1-2 *mm* in diameter) sampled from field by a modification of the ring method (Vargas and Hattori, to be published). Table 1 shows observed frequencies of each protozoa group. Since one gram of soil contained about 710 aggregates (1-2 *mm* in diameter), the number of cells in any group exceeded that of aggregates as shown in Table 2. However any protozoa groups did not occur in all of the aggregates. Flagellates occured in 80% of them even at the most frequent case.

The result suggests that protozoa cells can proliferate in one and the same aggregates but proliferateed cells cannot move freely

Table 1. Occurrence of each group of protozoa among soil aggregates

Protozoa group	Number of aggregates from which protozoa were found*
Amoeba	222 (67.3**)
Flagellates	266 (80.6)
Small ciliates	141 (42.2)
Colpoda sp.	150 (45.4)
Large ciliates	131 (39.6)

* The number of aggregates tested was 330.
** Figures in parenthesis indicate percentage.
Based on Vargas and Hattori (to be published).

Table 2. The numbers of cells of each protozoan group per *g*-soil

Protozoan group	Number of cells	Mean number per aggregate
Amoeba	1.23×10^5	172.3
Flagellates	2.73×10^4	38.2
Small ciliates	1.21×10^4	17.0
Colpoda spp.	5.72×10^3	7.4
Large ciliates	1.09×10^3	1.5

Based on Vargas and Hattori (to be published).

from aggregate to aggregate; probably their free movement among aggregates requires thick water film which is usually wanting in field condition.

2. Protozoan predation of bacterial cells in soil aggregates

Using sterilized aggregates we can study how protozoan cells predate bacterial cells in aggregates. Figure 17 shows *Collpoda sp.* attacks mainly bacterial cells in the outer part of aggregates and hardly do those in the inner part. This indicates that protozoan cells moves to attack bacteria in the large pores of the outer part but not in small pores in the inner part since the latter pores are smaller than protozoan cells. However this

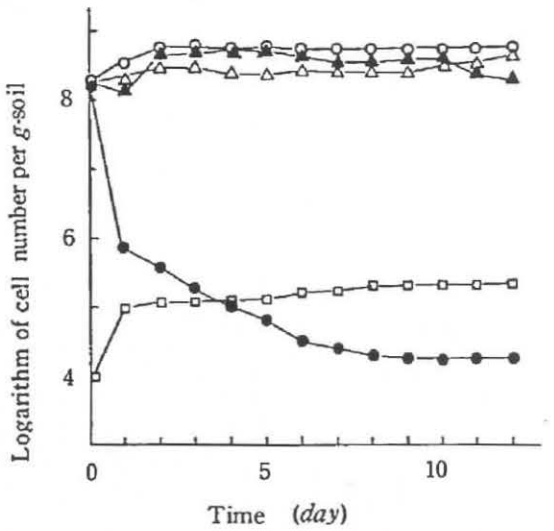

Figure 17. Predation by *Colpoda sp.* of *K. aerogenes* in sterilized aggregates.
Opened circles and triangles indicates bacterial numbers in the inner and the outer parts without the addition of protozoa. Closed circles and triangles indicate bacterial numbers in theouter and the inner parts with protozoan cells. Opened squares indicate protozoan number. Vargas and Hattori, 1986.

does not exclude that some protozoan cells can squeeze bacteria in small pores to some extend (Vargas and Hattori, in press).

Added protozoan cells have considered to be distributed randonly in large pores but the movement of protozoan cells from pore to pore is limited by water content in aggre-

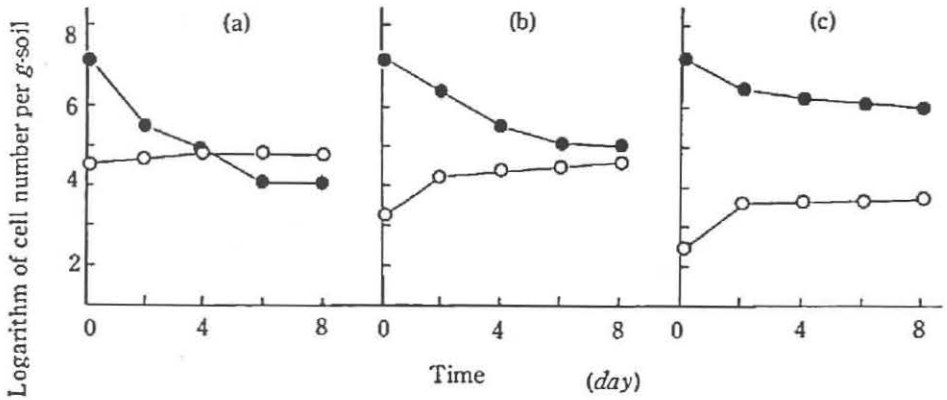

Figure 18. Effect of the initial population density of *Colpoda sp.* on its predation of *K. aerogenes*.
(a), (b), and (c) indicate the predation when different initial density of protozoan cells were added. Opened and closed circles indicate protozoan and bacterial numbers, respectively. Vargas and Hattori, 1986.

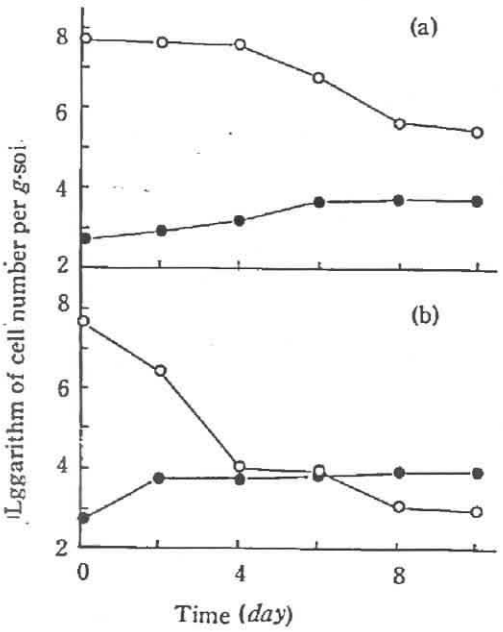

Figure 19. Effect of water content on the predation by *Colpoda sp.* of *K. aerogenes* in sterilized aggregates.
(a); water content is 60% of the maximum water holding capacity (W.H.C.) and (b); 80% of W.H.C.. Opened and closed circles indicate protozoan and bacterial numbers, respectively. Vargas and Hattori, 1986.

gates. Figure 18 shows that the decrease of bacterial number depend largely on the initial number of protozoan cells when water condition or the total volume of suspension added is kept at a level of 60 w.H.C.. That is, a larger part of bacterial cells may be attacked if number of protozoan cells is so large that we can find protozoa in every pore of the outer part; but only a small part of bacterial cells may be attacked if the number of protozoan cells added is so small that we can not find protozoa in many pores and water content is so small that protozoa can not move from pore to pore.

The above consideration is supported by the experiment in Figure 19. The figure indicates that, though the number of protozoan cells is small, protozoa can attack bacterial cells more effectively if a larger volume of water is supplied; under this condition protozoa can move from pore to pore and probably aggregate to aggregate as well.

VII Distribution of individual cells in microaggregates or at their microsites

We have considered microbial distribution in soil aggregates collectively in a sense. In this chapter we will try to get information on the distribution of individual cells.

1. Bacterial cells in microaggregates or on soil particles

Yamagishi (1968, 1986) dispersed soil in water by hand-shaking and aggregates or particles of various size were fractionated into several groups by sedimentation velocity in sterilized water, which repeated until freely suspended cells were mostly washed out. Then the mean size and the number of microaggregates were determined by Coulter counter. The number of microaggregates retaining cell(s) was estimated by incubating a definite volume of the microaggregates suspension on the albumin agar plate. We could thus obtain the ratios of microaggregates having cell(s) to all the microaggregates.

Figure 20 indicates the relationship

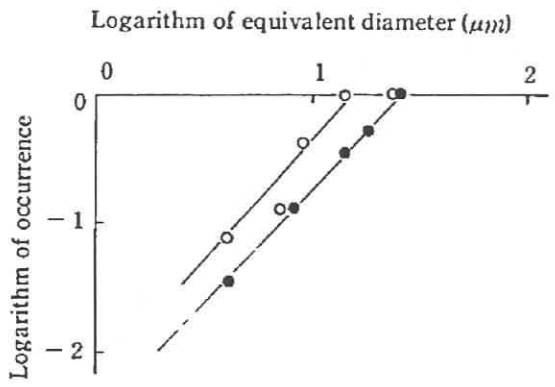

Figure 20. Occurrence of bacterial cells among soil particles or aggregates of various sizes. Opened and closed circles indicates fresh soil sample and air-dried sample. Based on Yamagishi's data, 1968.

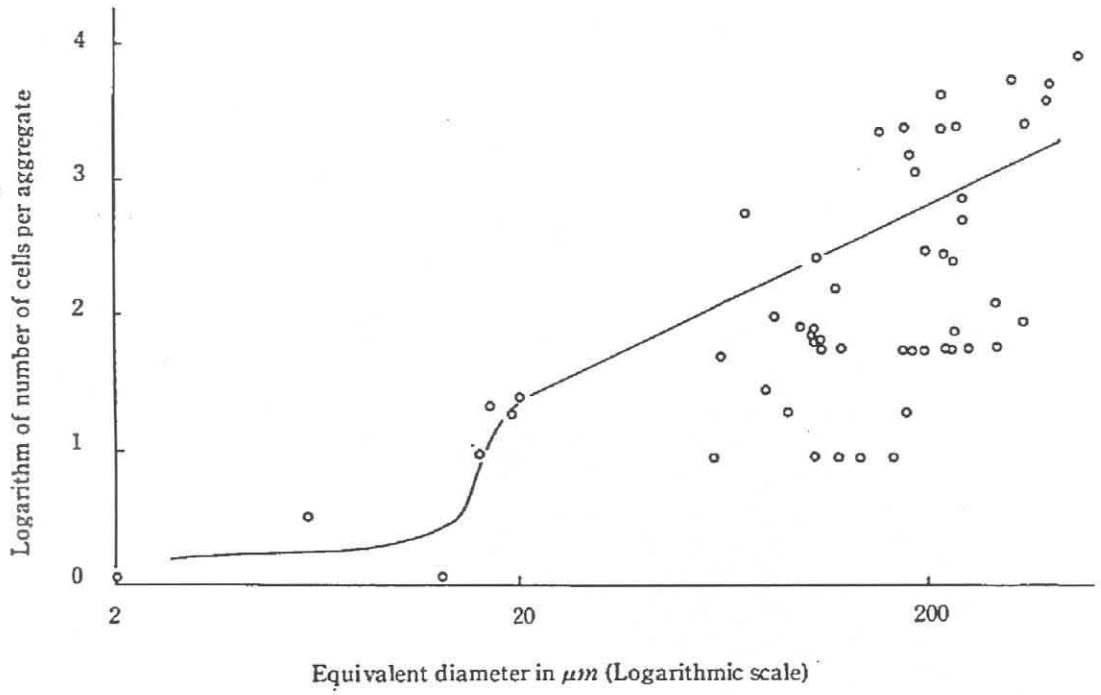

Figure 21. Effect of sonic oscillation on the plate count of bacteria retained by aggregates. Aggregates less than 20 μm in the eqivalent diameter were examined collectively as a group and the count indicates the mean value. Aggregates larger than 40 μm in the diameter were examined separately. Based on Yamagishi's data, 1969.

between the ratio and the mean equivalent diameter of microaggregates. She recognized linear relationships between the two variables. The result suggested bacterial cells existed randomly on the surface or in the inside of microaggregates. However there was a possibility that cells enclosed in the inside of aggregates may not grow to produce colonies. She therefore disrupted aggregates by sonic oscillation and determined the number of bacteria. The results are shown in Figure 21. The increase in the number of colonies is remakable with aggregates larger than 5 μm in diameter. It means that aggregates larger than this size contains pores in which bacterial cells are living.

2. Observation of thin soil sections by electron microscope

Electron microscopic photographs of thin sections of soil aggregates showed different morphological bacterial cells on the surface and in the inside of aggregates (Balkhi *et al.*, 1978; Foster *et al.*, 1983); probably Gram negative bacteria in the inside and Gram positive bacteria at the surface. Kilbertus (1980), using electron microscopic photographs of successive thin sections of aggregates, made a statistical study on the size distribution of pores in which bacterial cell(s) exist. According to his result most of cells in the inside were found in pores 1 to 3 μm in diameter and these bacterial cells were observed singly or in microcolonies of two or three cells.

3. Consideration of bacterial colony size in soil suspension

As to the distribution and the physiological state of bacterial cells in soil, Skinner presented an interesting consideration (1976).

According to the procedure original to Jones-Mollison soil was ground gently with

water and thus we can obtain information on the distribution of individuall cells in the suspension by microscopic examination. Quenouille described a note of Jones and Mollison's paper (1948); bacterial cells were distributed in colonies in a way according to a logarithmic series; this series is an expansion of the termin $(1-x)$

$$-\ln(1-x)=x+x/2+x/3+\ldots. x/n$$

The probability of finding colonies with 1, 2, 3, or n cells is given by the term x, $x/2$, $x/3$, or x/n, respectively.

Skinner (1976) considered that the logarithmic expression was not due to breaking up of large colonies by the grinding and mixing procedure needed to prepare agar films, but reflected with fair accuracy extent the distribution of individual cells in undisturbed soil. Thus he concluded that the growth of a single cell into a colony is subjected to severe limitation.

VIII Concluding remaks

Now we conclude that the structure of soil aggregates is very important in understanding of the distribution of various microorganisms in soil and of the control of their population changes. The living space of aggregates can be divided into two categories; one is the inner part which consists from smaller pores and the other is the outer part which consists of larger pores and the surface of aggregates. The critical size of pores dividing them into the two parts is estimated to be between 2.5 and 6 μm in the diameter of pore neck.

Bacterial population was constantly of high density in the inner part but fluctuate largely in the outer part. Notably the density of Gram negative bacteria was very high in the former part although their density was usually very low in the outer part under field conditions. Most of fungi were found mainly in the outer part but some group such as *Fusarium* was also in the inner part. Most of protozoa were also observed in the outer part.

The difference of the distribution and population change in aggregates between bacteria and other microorganisms can be interpreted largely by the size of pores in which microbial cells reside and by the retention of water in pores, that is, the capillary forces.

Although we supposed in earlier papers that becterial cells may be largely colonized in the small pores of the inner part, but observations of thin sections of soil showed that cells are distributed rather singly or in microcolonies consisting of only two or three cells. This result coincides with the idea that bacterial cells in soil are mostly in a state of non-growing.

XI. Summary

The pore system of soil aggregates was discussed in relation to microbial distribution and its change. The pore space was divided into two categories: the inner part and the outer part. The former consists of pores larger than 2.5-6 μm in the diameter of pore neck and the later consisting of those less than the critical size of pore neck. Bacterial cells lived constantly in the inner part but other micro-organisms reside in the outer part mostly. Such a distribution patterm is very important in understanding of microbial dynamics in soil.

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